

JAP20Rec'd PCT/PTO 16 MAY 2006

## AMENDED CLAIMS

[received by the International Bureau on 25 April 2005 (25.04.2005);  
original claims 1-26 replaced by amended claims 1-26 (4 pages)]

1. A chimeric gene comprising the following operably linked DNA:
  - (a) a plant-expressible promoter;
  - (b) a DNA region which when transcribed yields a double-stranded RNA molecule capable of reducing the expression of an essential gene of a plant sap-sucking insect, said RNA molecule comprising a first and second RNA region wherein:
    - (i) said first RNA region comprises a nucleotide sequence of at least 19 consecutive nucleotides having at least about 94% sequence identity to the nucleotide sequence of said endogenous gene ;
    - (ii) said second RNA region comprises a nucleotide sequence complementary to said at least 19 consecutive nucleotides of said first RNA region;
    - (iii) said first and second RNA region are capable of base-pairing to form a double stranded RNA molecule between at least said 19 consecutive nucleotides of said first and second region;
  - (c) optionally, a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of said plant.
2. The chimeric gene of claim 1, wherein said essential gene of said plant sap-sucking insect is selected from the group consisting of the genes encoding the following: a gut cell protein, a membrane protein, an ecdyson receptor, a vATPase, an amino acid transporter, a transcription factor, a peptidylglycine alpha-amidating monooxygenase; a cysteine protease, an aminopeptidase, a dipeptidase, a sucrase/ transglucosidase, a translation elongation factor, the eucaryotic translation initiation factor 1A, a splicing factor, an apoptosis inhibitor, a tubulin protein, an actin protein, an alpha-actinin protein, a histone, a histone deacetylase, a cell cycle regulatory protein, a cellular respiratory protein; a receptor for an insect-specific hormonal signal, a juvenile hormone receptor, an insect peptidic hormone receptor; a protein regulating ion balance in the cell, a proton-pump, a Na/K pump, an intestinal protease; an enzyme involved in sucrose metabolism, a digestive enzyme, a trypsin-like protease and a cathepsin B-like protease.
3. The chimeric gene of claims 1 or 2, wherein said double-stranded RNA silences the gene corresponding to the DNA sequence of any one of SEQ ID NO: 5 to 8, SEQ ID NO: 11 or SEQ ID NO: 12.

4. The chimeric gene of any one of claims 1 to 3, wherein between said first and second RNA region, a spacer region containing a plant intron is present.
5. The chimeric gene of any one of claim 1 to 4, wherein said essential gene has a portion which occurs with the same sequence or with at least 94 % sequence identity in homologous genes of several plant sap-sucking insects.
6. The chimeric gene of any one of claim 1 to 5, wherein said promoter is a constitutive promoter.
7. The chimeric gene of any one of claim 1 to 6, wherein said promoter is a vascular-specific or a phloem-specific promoter.
8. The chimeric gene of claim 7, wherein vascular- or phloem-specific promoter is selected from the group consisting of: the *rolC* or *rolA* promoter of *Agrobacterium rhizogenes*, the promoter of the *Agrobacterium tumefaciens* T-DNA gene 5, the rice sucrose synthase RSs1 gene promoter, the *Commelina* yellow mottle badnavirus promoter, the coconut foliar decay virus promoter, the rice tungro bacilliform virus promoter, the promoter of the pea glutamine synthase GS3A gene, the *invCD111* and *invCD141* promoters of the potato invertase genes, the promoter isolated from *Arabidopsis* shown to have phloem-specific expression in tobacco by Kertbundit et al (1991), the VAHOX1 promoter region, the pea cell wall invertase gene promoter, an acid invertase gene promoter from carrot, the promoter of the sulfate transporter gene *Sultr1;3*, the promoter of a plant sucrose synthase gene, the promoter of a plant sucrose transporter gene.
9. A plant cell, tissue, or a plant or a plant seed comprising the chimeric gene of any one of claims 1 to 8 or the double-stranded RNA molecule described in any one of claims 1 to 8.
10. A method to silence a gene of a plant sap-sucking insect, comprising applying to the feed of said plant sap-sucking insect unpackaged, naked dsRNA or siRNA which is targeted to an essential plant sap-sucking gene..
11. The method of claim 10, wherein said essential gene is any of the genes listed in claim 2 above.

12. The method of claim 10, wherein said application is by expression of a dsRNA chimeric gene in phloem cells of a plant.
13. A method to silence a gene in an plant sap-sucking insect, comprising: adding naked, unpackaged dsRNA or siRNA to the diet or feed of said plant sap-sucking insect, wherein said dsRNA or siRNA targets said gene..
14. A method of controlling plant sap-sucking insects, comprising expressing in the phloem of a plant dsRNA that targets an essential plant sap-sucking insect gene.
15. The method of claim 14 wherein said gene is a gene expressed at least in the intestine or in gut cells.
16. The method of claim 14 wherein said plant sap-sucking insect is an aphid or a whitefly.
17. A plant, comprising stably inserted in its genome, the chimeric gene of claim 1, so that said chimeric gene is expressed in the phloem or xylem of said plant.
18. A method of identifying gene function in a plant sap-sucking insect, comprising the step of applying naked, unpackaged dsRNA targeting a plant sap-sucking insect gene to the diet of said insect, and evaluating phenotypic or biochemical changes in said insect.
19. A method of identification of novel targets for insecticidal compounds, comprising the steps of: a) applying naked, unpackaged dsRNA or siRNA molecules to the feed or diet of a plant-sap sucking insect; b) analyzing which genes when silenced confer lethality to said insect, c) cloning and characterizing said genes thus analyzed; d) identifying compounds that disrupt or inactivate said gene or the RNA or protein encoded thereby; and e) contacting said compounds with said insect or feed or diet of said insect to confirm the pesticidal nature of said compound.
20. Phloem of a plant, comprising siRNA targeted to an aphid essential gene.
21. Phloem sap of a plant, comprising siRNA targeted to an aphid essential gene.

22. An aphid gene comprising the sequence of any one of SEQ ID NO:5 to 8, or SEQ ID NO:12.
23. The method of claim 18 or 19, wherein a cationic oligopeptide is mixed in the diet together with the dsRNA.
24. The method of claim 23, wherein said oligopeptide is a 12 amino acids poly-Arginine peptide.
25. The plant cell, tissue, plant or plant seed of claims 9 or 17, which also comprises a chimeric gene encoding a cationic oligopeptide.
26. The plant cell, tissue, plant or plant seed of claim 25, wherein said oligopeptide is a 12 amino acids poly-Arginine peptide.

bcs03-2008  
SEQUENCE LISTING

AP20 Recd PCT/PTO 16 MAY 2006

&lt;110&gt; Commonwealth Scientific and Industrial Research Organisation

Bayer BioScience NV

Waterhouse, Peter

Whyard, Steven

Van Rie, Jeroen

&lt;120&gt; Insect resistance using inhibition of gene expression

&lt;130&gt; BCS03-2008 W01

&lt;150&gt; US 60/520,306

&lt;151&gt; 2003-11-17

&lt;160&gt; 12

&lt;170&gt; PatentIn version 3.0

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